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INVESTIGATING THE RECENT INVASION OF SILVER CARP (*HYPOPTHALMICTHYS MOLITRIX)* INTO KENTUCKY LAKE, UTILIZING DIET AND LONG TERM DATA ANALYSIS

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Silver Carp (*Hypopthalmicthys molitrix*) have established populations throughout the Midwestern U.S and populations in Kentucky Lake have increased rapidly within the past decade. This project aims to understand potential impacts of Silver Carp on reservoir primary productivity utilizing diet and long term data analysis. Understanding the diet of Asian Carp specific to Kentucky Lake is of primary importance for understanding changes in long term patterns of Kentucky Lake. A total of 60 Silver Carp were sampled for diet analysis. Silver Carp diets consisted predominantly of phytoplankton (83.6% ± 0.4) and secondarily of zooplankton (16.4% ± 0.4). In addition, we analyzed long term data of Kentucky Lake utilizing 23 years of limnological data collected from consistent sampling sites. Environmental variables were compared before (1990-2004) and after (2005-2013) the Asian Carp population explosion utilizing non-metric multidimensional scaling (NMDS). To date, overall NMDS results show overlap between pre-and-post reservoir conditions. However, some patterns show important differences in limnological data (e.g. primary productivity and nutrient levels) and are being analyzed further. Whether the recent invasion of Silver Carp is negatively impacting Kentucky Lake is not yet clear. Understanding long term trends in the Kentucky Lake ecosystem is important to understanding the invasion ecology of Silver Carp.

NOTES

ASSESMENT OF CHYTRID FUNGUS (*Batrachochytrium dendrobatidis*) OCCURRENCE AND PREVALENCE IN PLETHODONTID SALAMANDERS ACROSS A FOREST DISTURBANCE GRADIENT IN SOUTHEASTERN KENTUCKY

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Chytridiomycosis is an infectious disease of amphibians caused by the aquatic fungal chytridiomycete *Batrachochytrium dendrobatidis* (Bd), an aquatic pathogen that has caused the decline or extinction of approximately 200 species worldwide. Relatively little research has been conducted regarding the distribution and prevalence of Bd within the highly biodiverse forest ecosystems of Appalachia. Resource extraction practices have been shown to cause physiological changes in amphibians that can result in an immune-compromised condition and increased susceptibility to disease. This study represents the first to examine the presence of chytrid infection within the plethodontid salamander assemblages of eastern Kentucky, an area where surface mining for coal and timber harvest have heavily impacted forest and aquatic ecosystems as a result of pollution, sedimentation, and habitat fragmentation; factors that are known stressors of many amphibian species. Our objective was to comparatively characterize the prevalence and infection intensity of Bd for plethodontid salamander assemblages within three different watershed disturbance treatments (surface mine-impacted, recently logged, and intact mature forest). We captured and skin swabbed 307 salamanders during 14 sampling sessions in spring 2013 and tested them for the presence of Bd using a quantitative PCR. Bd was detected in a variety of salamander species from all three treatments including 9 of 200 (4.5%) in control streams of intact forest, 6 of 93 (6.5%) in watersheds with recent (< 5 years ago) timber harvest, and 2 of 31 (6.5%) in streams impacted by surface mining. Although we found no differences in Bd prevalence among treatments ($p > 0.05$), our findings are the first to confirm the presence of Bd in amphibians of this region, including within one of Kentucky's few reference watersheds. Given the difficulty of capturing salamanders in surface mine-impacted streams and our low resultant sample size in this treatment, we recommend additional sampling be conducted in other similarly disturbed areas to more robustly assess differences in Bd occurrence and prevalence among different forest disturbance regimes.

NOTES

STRAIN DIVERSITY IN THE FECAL INDICATOR *ESCHERICHIA COLI*: IMPLICATIONS FOR ITS USE AS A PRODUCE PATHOGEN SURROGATE

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Contamination of food and water by pathogens is a substantial public health issue in the United States. One of the challenges in protecting the public against water and foodborne illness is understanding the routes of contamination. One suspected route of food contamination is irrigation water. Surface water and ground water sources are often utilized for the irrigation of crops, and fresh produce in particular. *Escherichia coli* are widely used as fecal indicator bacteria (FIB) and as a surrogate for pathogens for water quality and food safety research. Using *E. coli* as a surrogate for produce pathogens should aid in research to understand the sources of contamination and how these pathogens persist in the water, soil and on plant surfaces. A key step in using *E. coli* as a pathogen surrogate is to understand how this organism varies in the environment relative to pathogens so that the most representative surrogate can be selected. Research is needed to find superior surrogates for produce pathogens to provide researchers a more effective indicator of produce pathogens and to understand the role that irrigation water plays in the contamination of produce with pathogens.

In this study 63 *E. coli* isolates were selected from a pool of 1,346 i solates previously taken from poultry, swine, dairy, and agriculturally impacted surface water sources (Cook et al, 2011). These 63 environmental *E.coli* isolates, the produce related pathogens *Salmonella*, *Listeria*, *E. coli* O157:H7 and a common quality control *E.coli* strain (EC 25922) were evaluated for factors that have been commonly linked to the survival and transmission of produce associated pathogens. These factors include biofilm formation, curli expression, and growth rate. These assays were completed when isolates were grown in a nutrient rich environment (Luria-Bertani broth [LB]) and a nutrient poor environment (Lettuce lysate + minimal salts [LM]). These characteristics are thought to play major roles in the ability of pathogens to survive and persist in the soil and on plant surfaces.

Of the 63 isolates, only six were determined to be negative for curli expression, while *E.coli* O157:H7 was the only pathogen that was positive. *Salmonella* and EC 25922 were inconclusive. Growth rates were considerably higher in LB versus LM which was expected, however when analyzing LM growth rates, EC 25922 had one of the highest growth rates out of all isolates and *Salmonella* one of the lowest. *Salmonella* biofilm formation was approximately 15 times higher than that of the common quality control strain EC 25922 when grown in LM. When comparing EC 25922, *E.coli* O157:H7 and *Listeria* to the 63 i solates, the 63 isolates had higher overall biofilm formation when grown in LM than when grown in LB. Six of the 63 isolates had biofilm formation that was three times higher than EC 25922 (Figure 1).

These data exemplify the diversity in isolates from environmental sources, and that *E.coli* quality control strains such as EC 25922 may not be appropriate indicators of pathogens because of the vast differences in how they behave in environmental situations. Using these data, and continued characterization of the isolates (carbon source utilization, genotypic data, soil and lettuce adhesion), isolates will be selected for future greenhouse experiments. These isolates will then be applied to soil and lettuce leaf substrates via spray or drip irrigation water, and isolate survival will be monitored over time. This research will provide science based information on the differences in *E. coli* isolates from multiple environmental sources, the selection of enhanced produce pathogen surrogates, and the potential of produce contamination via contaminated irrigation water.

Figure 1. Comparison of Biofilm production by top preforming environmental isolates compared to the industry quality control strain EC 25922.